reductions by PP2 and U7 may have been generated via different mechanisms, given that the two drugs had varying effects on sperm incorporations and pronuclear differentiations. Moreover, confocal imaging revealed Ca2+ oscillations were blocked by U7 but not by PP2. Collectively, such data fail to support the view that SFK signaling is required for either GVBD or for initiating fertilization-induced Ca2+ oscillations in *Cerebratulus* and instead suggest that PP2-mediated inhibitions of polar body formation and cleavage involve undetermined drug effects on processes other than oscillation generation.

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Program/Abstract # 342
The mammalian Doublesex homolog DMRT1 controls the mitosis versus meiosis decision in males
David Zarkower[a], Clinton K. Matson[a], Mark W. Murphy[a], Anthony D. Krentz[a], Shosei Yoshida[b], Vivian J. Bardwell[a]
[a]*Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455, USA*
[b]*National Institute of Basic Biology, Okazaki, Aichi 444-8787 Japan*

Germ cells are uniquely capable of undergoing either mitotic divisions, like other cells, or meiotic divisions that permit gametogenesis. In mammals meiosis is triggered by retinoic acid (RA), which activates genes including the meiotic inducer *Stra8*. Fetal males avoid meiosis by degrading RA in the fetal testis. When meiosis begins in males at puberty it requires RA and *Stra8*, but how these are controlled in spermatogonia has been unknown. We have found that the Doublesex-related transcription factor DMRT1 determines whether spermatogonia undergo mitosis or initiate meiosis. Spermatogonia lacking DMRT1 have abnormally active RA signaling and prematurely enter meiosis, independent of the normal spermatogenic cycle. Chromatin immunoprecipitation and other approaches show that control of meiotic initiation by DMRT1 involves direct transcriptional regulation of key RA metabolic enzymes and *Stra8*. Analysis of vitamin A depleted animals that lack RA reveals that DMRT1 also controls at least one retinoid-independent meiotic inducer. These results establish DMRT1 as an essential and direct regulator of the mitosis versus meiosis switch. The DM domain gene family to which DMRT1 belongs is deeply conserved in metazoan sexual regulation, and thus our findings also may have implications for meiotic control outside of mammals.

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Program/Abstract # 343
The RNA-binding protein Nanos2 is required to maintain spermatogonial stem cells
Aiko Sada[a], Atsushi Suzuki[b], Hitomi Suzuki[c], Yumiko Saga[a,d]
[a]*Dept. of Genetics, SOKENDAI, Mishima, Shizuoka, Japan*
[b]*IRC, Yokohama National Univ., Hodogaya-ku, Yokohama, Japan*
[c]*Dept. of Biological Sciences, Tokyo Univ., Bunkyo-ku, Tokyo, Japan*
[d]*Division of Mammalian Development, NIG, Mishima, Shizuoka, Japan*

In mice, spermatogenesis is initiated from a small number of stem cells belonging to undifferentiated spermatogonia. However, it remains unclear 1) which types of spermatogonia actually act as the stem cells and 2) how is the stem cell function regulated. Nanos, a zinc-finger RNA-binding protein, has been proposed as a conserved factor for germline stem cell function. In adult testes, Nanos2 is predominantly expressed in a subset of undifferentiated spermatogonia. However, the majority of *Nanos2*-null germ cells die by apoptosis before birth, hindering functional studies of Nanos2 during spermatogenesis. With the use of transgenic mouse strategies, I found that the RNA-binding protein Nanos2 is a key regulator for the maintenance of spermatogonial stem cells. Lineage-tracing analyses revealed that *Nanos2*-expressing spermatogonia self-renew and generate the entire spermatogenic cell lineage. Conditional disruption of postnatal *Nanos2* depleted spermatogonial stem cell reserves, whereas mouse testes in which *Nanos2* had been overexpressed accumulated spermatogonia with undifferentiated, stem cell-like properties. Thus, Nanos2 is expressed in self-renewing spermatogonial stem cells and maintains the stem cell state during murine spermatogenesis.

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Program/Abstract # 344
Stage-specific expression of the homeodomain protein Cux1 in Sertoli cells and spermatids during spermatogenesis
Tony N. Jelsma[a], Melissa R. Kroll[a,b], Engela S. Viss[a,b], Jonathan Lamb[b], Joy Horstman[a,b], Alexander Powell[a,b], Andrea VanWyk[b], Kaarlo Hinkkala[a,b], Aaron Taylor[b], Gregory VandenHeuvel[b]
[a]*Dept. of Biology, Dordt College, Sioux Center, IA, USA*
[b]*Dept. of Anatomy and Cell Biology, Univ. Kansas Med. Center, Kansas City KS, USA*

The homeodomain protein Cux1 exists as multiple isoforms. The 200 kDa Cux1 protein is highly expressed in the developing kidney, where it functions to regulate cell proliferation. A 55 kDa Cux1 isoform is expressed exclusively in the testes. Transgenic mice ectopically expressing the 200 kDa Cux1 protein develop transient multiorgan hyperplasia, including the testes. We determined the pattern and timing of Cux1 protein expression in the developing testes. Cux1 expression was continuous in Sertoli cells of prepubertal testes, but became cyclic when spermatids appeared. In mature mice, Cux1 was highly expressed only in round spermatids at stages IV–V of spermatogenesis, in both spermatids and Sertoli cells at stages VI–X, and only in Sertoli cells at Stage XI. In Cux1 transgenic mice there were significantly fewer tubules expressing Cux1 in both Sertoli cells and spermatids and significantly more tubules expressing Cux1 in either spermatids or Sertoli cells. Moreover, Cux1 was not expressed in proliferating cells in testes from either wild type or transgenic mice. Thus, unlike the role of the somatic form of Cux1 in cell proliferation, the testis-specific form of Cux1 is not involved in cell division and appears to play a role in signaling between spermatids and Sertoli cells.

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Program/Abstract # 345
Inhibitory action of *Xenopus* dicalcin on sperm–egg interaction during fertilization
Naofumi Miwa[a], Motoyuki Ogawa[b], Yoshiki Hiraoka[c], Ken Takamatsu[a], Satoru Kawamura[d]
[a]*Dept. of Physiol., Toho Univ., Tokyo, Japan*
[b]*Dept. of Med. Educ., Kitasato Univ., Kanagawa, Japan*
[c]*Dept. of Anat., Keio Univ., Tokyo, Japan*
[d]*Grad. Sch. Frontier Biosci., Osaka Univ., Osaka, Japan*

To contribute to the study of sperm–egg interaction in the course of fertilization, we have isolated and characterized *Xenopus* dicalcin in *Xenopus* eggs. *Xenopus* dicalcin is localized markedly in the egg-coating envelope (called vitelline envelope; VE), and exhibits a Ca^{2+}-dependent binding to two glycoproteins that constitute polymeric filaments of VE. Since these VE glycoproteins are considered to function as sperm-receptors, we examined the effect of dicalcin on sperm–VE binding, sperm–VE penetration, and fertilization *in vitro*. Preincubation of *Xenopus*

eggs with recombinant dicalcin reduced the number of sperm that bound to VE as well as the efficiency of fertilization. By contrast, suppression of intrinsic dicalcin by preincubation with anti-dicalcin antibody increased both efficiencies. Furthermore, in our unique penetration assay, recombinant dicalcin inhibited sperm–VE penetration significantly. To further characterize this observation, we investigated the action of dicalcin on sugar distribution within the VE. Exogenously applied dicalcin increased *in vivo* lectin-reactivity in the VE, accompanied by an increase in the lectin-reactivity of isolated glycoprotein. These results suggested that dicalcin binds to VE glycoproteins and alters the pattern of sugar presentation, and thereby inhibits sperm–egg interaction.

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Program/Abstract # 346
Roles of hypoxic response genes in *Drosophila* primordial germ cell development
Elizabeth M. Asque, Jo Anne Powell-Coffman, Clark R. Coffman
Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA, USA

The cellular responses that allow a cell to survive and adapt to hypoxic stress (low oxygen) are largely conserved. The Hypoxia Inducible Factor transcription factors (HIFs) are the primary transcription factors mediating responses to hypoxic stress. HIFs are composed of alpha and beta subunits. HIFalpha is only stable in hypoxic conditions. The pathway for oxygen-dependent degradation of HIFalpha includes a prolyl-hydroxylase (PHD) and the VHL E3 ligase. The *Drosophila* homologs of HIFalpha, HIFbeta, PHD, and VHL are encoded by the *similar*, *tango*, *fatiga*/*Hph*, and *Vhl* genes, respectively. Previous studies have demonstrated that *similar* has roles in *Drosophila* tracheal development as well as border cell migration. Our research team utilizes *Drosophila* germ cell development as a model for studying cell migration and programmed cell death. The working hypothesis for this project is that primordial germ cell development will be responsive to hypoxic stress. Initial results using wild-type embryos have shown that exposure to hypoxia impacts germ cell migration and/or programmed cell death. We are following up these observations to determine if the key components of the hypoxic response pathway are necessary for germ cell migration and programmed cell death during *Drosophila* development.

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Program/Abstract # 347
A crucial role for lipid phosphorylation in WntD-mediated primordial germ cell migration
Mark A. McElwain, Dennis C. Ko, Michael D. Gordon, Roel Nusse
Department of Developmental Biology, Stanford University, Stanford CA, USA

Signaling pathways such as those controlled by the Wnt family of secreted ligands control many aspects of embryonic development and adult homeostasis. Often, the same pathway is used repeatedly to accomplish different tasks, raising the question of how these pathways are regulated in different biological contexts. Significantly more is known about β-catenin-dependent mechanisms of Wnt signaling than β-catenin-independent mechanisms. Therefore, our previous demonstrations that the *Drosophila* ligand WntD utilizes a β-catenin-independent pathway to control both dorsal/ventral patterning and primordial germ cell (PGC) migration led us to examine the mechanism of WntD signal transduction. We undertook a suppressor screen to identify the WntD receptor and other downstream components and discovered that loss of either *CG16708*, a putative ceramide kinase, or *CG31873*, a putative multi-substrate lipid kinase, suppresses WntD-induced lethal dorsalization. Additionally, embryo homozygous mutant for both genes display a *wntD* mutant-like phenotype in primordial germ cell migration. These and future studies could help reveal a model of how lipid metabolism controls PGC migration.

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Program/Abstract # 348
Prenylation-deficient heterotrimeric G protein gamma subunits reveal GPCR-mediated signaling events in vivo
Tim Mulligan, Steve Farber
Carnegie Inst., Baltimore, MD, USA

Geranylgeranylation is a post-translational process involving addition of a polyunsaturated lipid to proteins containing a carboxy-terminal CaaX motif. One family of geranylgeranylated proteins, the heterotrimeric G protein gamma (γ) subunits, is essential for the transduction of signals emanating from G protein coupled receptors (GPCR). To better understand the GPCR-mediated events involved in zebrafish development, we investigated the ability of Gγ subunits with mutated CaaX motifs to disrupt heterotrimeric G protein signaling when expressed in developing larvae. Our studies reveal that prenylation-deficient versions of Gγ subunits have the ability to disrupt GPCR signaling by altering the subcellular localization of signaling components. The disruption induced by expressing prenylation-deficient Gγ subunits ubiquitously or in primordial germ cells (PGC) manifests as a loss of directional PGC migration. The majority of Gγ subunits have the ability to disrupt PGC migration in the prenylation-deficient form, but only a distinct subset of wild type Gγ subunits have the ability to reverse this semi-dominant negative effect. To understand the roles that Gγ protein domains have in contributing to differences in Gγ signaling capacity in vivo, we constructed Gγ chimeras with swapped middle, N- or C-terminal domains. Analysis of these chimeras demonstrated that multiple regions and motifs within these regions influence the ability of Gγ to mediate the signaling pathways necessary for directional PGC migration. Our study of prenylated proteins involved in signal transduction contributes to the understanding of how lipids mediate development.

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Program/Abstract # 349
Hold on: Females modulate sperm release in *Drosophila melanogaster*
Margaret C. Bloch-Qazi
Dept. of Biol., Gustavus Adolphus College, St. Peter, MN, USA

Among species with internal fertilization, sperm fate is influenced by interactions with the female reproductive tract. The nature of these interactions is likely to be affected by the female's environment and physical condition although the extent of the effect(s) is poorly-understood. *Drosophila melanogaster* is a useful model system for examining female influence on sperm fate due to the ease with which the female's environment can be modified as well as the detailed understanding of associated reproductive processes such as gameto-genesis, mating and fertilization. A female's ability to regulate the release of sperm from storage sites was examined under two sub-optimal, but commonly experienced, conditions: limited exposure to fresh oviposition sites and increasing age. Females exposed infre-quently to fresh oviposition media became sperm depleted at a slower rate than did females exposed frequently to fresh media. Lower rates of depletion corresponded with preferential sperm retention within one type of storage structure, the seminal receptacle.